that this combination of three different tests gives the best overall concordance with rodent carcinogenicity. It is well known that the Ames test gives only just over 50% concordance with rodent carcinogenicity, but it is still extensively used. This is because it has a well-defined endpoint that one can readily understand in biological terms, i.e., genotoxicity. However, there is overwhelming evidence to show that enzymes of the cytochrome P450 superfamily are involved in the metabolism and toxicity of most (~90%) chemicals. P450s play a pivotal role in toxicity and carcinogenicity, and the COMPACT system is designed to identify P450-mediated metabolism and metabolic activation. Although this system (*3*) was originally based solely on the structures of known P450 substrates, we have now generated full three-dimensional structures (*4*) of the mammalian enzymes themselves (including human isoforms) which agree closely with experimental findings. However, we are also aware that there are other mechanisms of carcinogenicity that do not require P450 activation, and structure alert systems can be useful in identifying direct-acting carcinogens, for example (*1,5*).

Nongenotoxic carcinogens are not so easy to predict but we are elaborating models to identify chemicals involved in peroxisome proliferation and other activation pathways such as ß-lyase cleavage. Eventually there will be a battery of tests in place, which we hope will adequately assess the likely risk to *man* from exposure to foreign compounds; so models of human enzymes and receptors which may mediate potentially carcinogenic events will be important. What is crucial is the determination of how readily a chemical is metabolized and whether any reactive intermediates (ROS or metabolites) are sufficiently long-lived to cause irreparable DNA damage. There may well be short-term test procedures developed which can assess these factors *in vitro*, but computer-based systems can be just as accurately predictive; however, these computer systems do not require synthesis of the chemical, are extremely rapid, and, consequently, relatively inexpensive. We appreciate that traditional toxicologists may have been suspicious of replacing biological tests with computer predictions, but there is evidence that attitudes are changing.

One reason for publishing our retrospective study of the 40 NTP chemicals was to show that it is possible for a combination of tests to give reasonable concordances with rodent carcinogenicity, and we did not anticipate that moderating the equivocal results of the rodent study in the light of the pathology report presented at the NTP conference would be controversial. Our *EHP* paper was independently refereed and, as our use of modified equivocal results (reported at the meeting) was not questioned by the referees, one can only presume that NIEHS (which publishes *EHP* and also organized the 1993 conference) did not find this contentious. The problem regarding equivocals is well known, and these are obviously difficult to assess by predictive systems which, in general, do not equivocate. In fact, some systems (e.g., TOPKAT, CASE) tend to exclude equivocal results in the rodent assay when they validate their methods. At the NIEHS conference in 1993 there was lengthy discussion about equivocals, and the prevailing doctrine from NTP was to regard these as negatives, although there was not universal agreement for this view among the delegates. During the presentation of the pathology results it was indicated that a few of the equivocals could be interpreted, on histopathological evidence, as being weakly positive. It did not seem unreasonable to us in our retrospective analysis to take into account the views of the NIEHS pathologist who conducted the examinations. However, if one excludes the equivocals, the concordance between COMPACT and the rodent assay becomes 70% (21/30), which is not much different from the concordance one gets from regarding three of the equivocals as positives. If one regards all of the equivocals as positives, the concordance between COMPACT and the rodent carcinogenicity is 69% (25/36), whereas taking them as negative lowers the concordance further to 64% (23/36). However, it should be noted that if these three equivocals are regarded as positive, most (if not all) of the predictive tests show a similar improvement.

The use of metal ion redox potentials for providing some estimate of carcinogenicity is not currently part of COMPACT, but it is of interest to show that physicochemical parameters may be employed to try to predict the potential carcinogenicity of inorganic compounds. Likewise, Hazardexpert is not part of COMPACT, but the two tests are fairly complementary in the comparisons we have made to date (*1,6*).

In our *EHP* paper we provide explanations of the results for each chemical, including possible reasons why some of these were discordant with the rodent assay. However, we have not altered our results in the light of this additional biochemical knowledge, especially when the predictive methods (e.g., Ames test, Ashby structural alert, etc.) were able to predict correctly the outcome of the rodent carcinogenicity study for those chemicals. The purpose of these developments of predictive systems is to be able to decrease the number of animal experiments and the time required for the safety evaluation of chemicals that are destined for human exposure, and we believe that a combination of several systems represents the best way to achieve this (*6*), with the consideration of P450-mediated pathways of activation and detoxication being most important. Our *EHP* paper merely attempts to show how a combination of systems might work, but we would appreciate advice from a statistician on how to "weight" such tests: perhaps Joe Haseman could help us.

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References

1. Brown SJ, Raja AA, Lewis DFV. A comparison between COMPACT and Hazardexpert evaluations for 80 chemicals tested by the NTP/NCI rodent bioassay. Alter Lab Anim 22:482–500 (1994).
2. Lewis DFV, Ioannides C, Parke DV. Computer-optimised molecular parametic analysis of chemical toxicity (COMPACT). In: Proceedings of the conference on predicting chemical carcinogenesis in rodents, 24–25 May 1992, Research Triangle Park, North Carolina. Research Triangle Park, NC:National Institute of Environmental Health Sciences, 1992; 45–49.
3. Lewis DFV, Ioannides C, Parke DV. Validation of a novel molecular orbital approach (COMPACT) to the safety evaluation of chemicals by comparison with Salmonella mutagenicity and rodent carcinogenicity data evaluated by the US NCI/NTP. Mutat Res 291:61–77 (1993).
4. Lewis DFV, Moereels H, Lake BG, Ioannides, Parke CD. Molecular modelling of enzymes and receptors involved in carcinogenesis: QSARS and COMPACT-3D. Drug Metab Rev 26:261–285 (1994).
5. Lewis DFV. Computer-assisted methods in the evaluation of chemical toxicity. Rev Computat Chem 3:173–222 (1992)
6. Lewis DFV. Comparison between rodent carcinogenicity test results of 44 chemicals and a number of predictive systems. Regul Toxicol Pharmacol 20:215–222 (1994).

Drinking Water and Leukemia

Cohn et al. recently expanded (*EHP* 102:556–561) on an earlier ecological study (*1*) of leukemia and drinking water in northern New Jersey. The initial study suggested an association between volatile organic hydrocarbon (VOC) contamination of drinking water and increased risk of leukemia among females (but not males). The authors concluded that 1) the appearance of an association for females only

could not be explained; 2) the limitations of the ecologic study design precluded inferences regarding causality; and 3) the case–referent study would be a useful follow-up in assessing the public health significance of their observations.

The latest study by Cohn et al., while rich in methodological details and rigorous in discussion of its limitations, does not really clarify the discussion of potential health effects of low-level VOC [particularly trichloroethylene (TCE) and perchloroethylene (PCE)] exposures. The addition of 48 municipalities to the study area, extension of the observed period of disease incidence through 1987, and inclusion of non-Hodgkin's lymphomas (NHLs) only increased the size of the study; these changes did not mitigate the weaknesses of the ecologic design. Cohn et al. suggested that since their unit of analysis was the municipality, in which virtually all residents could be presumed to have the same average exposure, their analysis approximated that of a crude individual-level analysis. We believe that this suggestion is based on a misinterpretation of Greenland and Morgenstern (*2*); these authors observed that ecologic bias cannot occur when the geographical units being compared are either 100% exposed or 100% unexposed. In the study by Cohn et al., this condition cannot be said to obtain, as they implicitly recognized in their discussion of exposure misclassification.

In any case, even in the extreme situation described by Greenland and Morgenstern, the analysis approximates a *crude* individual-level analysis, which has not taken individual-level confounding factors, both measured and unmeasured, into account. Such factors (e.g., socioeconomic, occupational, and lifestyle indicators) may still seriously bias the effect estimate in an ecologic study even when they do not appear to be confounders at the ecologic level, especially when the range of estimated group exposure levels is narrow and the effect of exposure on risk appears to be weak (as is true in the study by Cohn et al.) (*2*). Moreover, Cohn et al. analyzed their data in the presence of an apparent effect modifier, sex, which they intended to address as their fourth study hypothesis. Unfortunately, they left unanswered the question of why the effects observed in their study should be specific to females or males only.

More importantly, a closer look at the published results of laboratory and epidemiology studies of TCE and PCE leads to more consistent and persuasive conclusions. TCE was associated with a small increase in immunoblastic lymphosarcoma in two rodent studies, one using the inhalation route (100, 300, and 600 ppm), and one using the oral route (50 or 250 mg/kg) (*3,4*). In neither study, however, was the increase significantly dose related and in neither was the increase considered significant given the high variability of background incidence in control animals. No other rodent bioassays of TCE have resulted in increased risk of lympho-hematopoietic cancer (*5*). PCE was associated with increased incidence of mononuclear cell leukemia in Fisher 344/N rats in a National Toxicology Program bioassay (*6*), using inhalation exposures of 200 or 400 ppm. The significance of this finding was questioned, however, by the U.S. EPA's Science Advisory Board (*7*) because of the "consistently higher control rates of this tumor type in the study laboratory, coupled with widely variable incidence (in comparison with other NTP laboratories)." The evidence for TCE and PCE as rodent lympho-hematopoietic carcinogens is therefore relatively weak, especially since neither has been shown to exhibit mutagenic or genotoxic activity (*5,8*).

Cohn et al. suggested that occupational studies of TCE and PCE exposures "were small, had short follow-up times, and were based on mortality" (p. 560). This inaccurately characterizes these studies, which we believe have provided excellent evidence with which to judge the likely human carcinogenicity of fairly high-level exposure to these materials. Axelson et al. (*9*) recently published the results of an incidence study of cancer among TCE-exposed workers in Sweden (with exposure documented by urinary metabolite measurement and incidence ascertained from 1958 through 1987). No cases of lympho-hematopoietic cancer were observed among the 249 females included in the study (expected number not reported); among 1421 male workers, 5 NHLs were observed, with 3.2 expected (standardized incidence ratio = 1.56, 95% CI, 0.51–3.64). Average exposure levels in this cohort were thought to be on the order of 30–50 ppm in air (with the male workers having longer average duration of exposure than the females); however, the urinary metabolite measurement protocol was not described, and it is conceivable that the biomonitoring data underestimated the actual exposures. Axelson et al. concluded that "the cancer risk to humans from TRI [TCE] exposure is rather small, if any, under the circumstances that have prevailed when using TRI" (*9*: p. 561).

In a large study of aircraft maintenance workers exposed to intermittent TCE levels as high as 400 ppm, U.S. National Cancer Institute investigators followed approximately 7000 subjects from 1952 through 1982 (*10,11*). Among male workers, they observed 9 leukemia deaths compared with 13.1 expected [standardized mortality ratio (SMR) = 69, 95% CI, 31–130] and 10 NHL deaths, compared with 9.8 expected (SMR = 103, 95% CI, 49–189). Among female workers they observed 2 leukemia deaths, compared with 1.9 expected (SMR and CI not calculated) and 4 NHL deaths, compared with 1.4 expected (SMR = 286, 95% CI, 78–731). The small excess of NHL deaths among female workers was not consistently associated with increasing cumulative exposure. Spirtas et al. concluded that "it is only possible to suggest, at this time, that occupational exposure to TCE probably does not pose a strong carcinogenic risk for man" (*10*: p. 528).

The most recent analysis of mortality in a large cohort of dry-cleaning workers, by Ruder et al. (*12*), which was an update of the study by Brown and Kaplan (*13*), studied 1690 workers with follow-up from 1940 through 1990. In a subcohort of workers thought to have been exposed only to PCE, only 2 deaths due to lympho-hematopoietic cancer were observed (about 4.1 expected, SMR = 49, 95% CI, 6–177). n the remainder of the cohort, exposed to PCE and other dry-cleaning solvents, 7 deaths due to lympho-hematopoietic cancer were observed (about 9 expected, SMR = 78, 95% CI, 31–161). These observations are consistent with those of Blair et al. (*14*), who studied a cohort of 5365 dry-cleaners from 1948 through 1979. They observed a small excess of all lympho-hematopoietic cancer deaths, 24 observed versus 20.0 expected (SMR = 120, 95% CI, 80–180). This excess appeared to be related to level of exposure, but only among white males; among females, deaths in this cancer category were limited to the lowest exposure group. Quantitative PCE exposures were not estimated for the workers in the two studies described above. Such exposure has been reported to vary between 28.2 and 88.2 ppm (time-weighted average) in a study of 67 dry-cleaning shops in the United States (*15*).

Cohn et al. assigned estimated VOC exposure levels in their study using categories of < 0.1 ppb, 0.1–5.0 ppb, and > 5.0 ppb. The animal bioassay data and the human occupational data, obtained from large studies of workers exposed daily to TCE or PCE at air concentrations of parts per ***million***, do not support the existence of an association between these exposures and lympho-hematopoietic cancer. To believe that the exposures described by Cohn et al., in the low parts per ***billion***, could be causally associated with these diseases would be tantamount to standing the concept of dose response on its head. Cohn et al. suggested that human carcinogenicity "could involve different potencies and different organs or cell types from

those in the rodent studies" (p. 560), but they offered no suggestions as to the possible mechanisms that might underlie such differences, nor did they adequately address the substantial body of human epidemiologic data indicating no excess risk of lympho-hematopoietic cancer among workers exposed to TCE and PCE levels approximately three orders of magnitude greater than the New Jersey residents studied.

In summary, we conclude that the evidence from high exposure-level human and animal studies strongly suggests that there is not likely to be any increase in the risk of lympho-hematopoietic cancer in populations exposed to very low levels of TCE or PCE in drinking water. The results obtained by Cohn et al. are perhaps more likely explainable by the limitations of the ecologic study design. While fully supporting all efforts that have been and continue to be made by chemical manufacturers and users to reduce emissions into the environment, we hope that this brief review will encourage Cohn et al. and other public health investigators to view the results of ecological studies of low-level VOC exposures in a wider perspective.

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References

1. Fagliano J, Berry M, Bove F, Burke T. Drinking water contamination and the incidence of leukemia: an ecologic study. Am J Publ Health 80:1209–1212 (1990).
2. Greenland S, Morgenstern H. Ecological bias, confounding, and effect modification. Int J Epidemiol 18:269–274 (1989).
3. Maltoni C, Lefermine G, Cotti G. Experimental research on trichloroethylene carcinogenesis. Arch Res Ind Carcinog 5: (1986).
4. Maltoni C, Lefermine G, Cotti G, Perino G. Long-term carcinogenicity bioassays on trichloroethylene administered by inhalation to Sprague-Dawley rats and Swiss and B6C3F1 mice. Ann NY Acad Sci 534:316–342 (1988).
5. ECETOC. Trichloroethylene: assessment of human carcinogenic hazard. Technical report no. 60. Brussels:European Centre for Ecotoxicology and Toxicology of Chemicals, 1994.
6. U.S. NTP. Toxicology and carcinogenesis of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F$_1$ mice (inhalation studies). NTP technical report 311, NIH publication no. 86-2567. Research Triangle Park, NC:National Toxicology Program, 1986.
7. U.S. EPA. Health effects assessment of perchloroethylene. EPA SAB-EHC-91-013. Washington, DC:Environmental Protection Agency, 1991.
8. ATSDR. Toxicological profile for tetrachloroethylene. Atlanta, GA:Agency for Toxic Substances and Disease Registry, 1993.
9. Axelson O, Selden A, Andersson K, Hogstedt C. Updated and expanded Swedish cohort study on trichloroethylene and cancer risk. J Occup Med 36:556–562 (1994).
10. Spirtas R, Stewart PA, Lee JS, Marano DE, Forbes CD, Grauman DJ, Pettigrew HM, Blair A, Hoover RN, Cohen JL. Retrospective cohort study of workers at an aircraft maintenance facility. I. Epidemiological results. Br J Ind Med 48:515–530 (1991).
11. Stewart PA, Lee JS, Marano DE, Spirtas R, Forbes CD, Blair A. Retrospective cohort study of workers at an aircraft maintenance facility. II. Exposures and their assessment. Br J Ind Med 48:531–537 (1991).
12. Ruder AM, Ward EM, Brown DP. Cancer mortality in female and male dry-cleaning workers. J Occup Med 36:867–874 (1994).
13. Brown DP, Kaplan SD. Retrospective cohort mortality study of dry cleaner workers using perchloroethylene. J Occup Med 29:535–541 (1987).
14. Blair A, Stewart PA, Tolbert PE, Grauman D, Moran FX, Vaught J, Rayner J. Cancer and other causes of death among a cohort of dry cleaners. Br J Ind Med 47:162–168 (1990).
15. Materna BL. Occupational exposure to perchloroethylene in the dry cleaning industry. Am Ind Hyg Assoc J 46:268–273 (1985).

Response

We thank Dr. Ramlow and Mr. Bloemen for their comments on our epidemiologic study (*EHP* 102:556–561) of low-level exposures to perchloroethylene (PCE) and trichloroethylene (TCE). Our study found associations between PCE and TCE in drinking water and the incidence of leukemia and non-Hodgkin's lymphoma (NHL) in both sexes, especially among females. We believe that our study is an important contribution to the weight of evidence concerning possible hazards of these uniquitous chemicals for the general population.

Ramlow and Bloemen take issue with the design of our study, citing the usual limitations and caveats of ecologic studies. (They note that we also cited these caveats.) However, our study did not use a classic ecologic design. In a true ecologic study, exposures are expressed as proportions or averages of aggregate groups, such as the percentage completing high school in a municipality. In this example, an individual either finished high school or did not. In contrast, we assigned the exposure category of each study subject according to the level of contamination in the water utility serving the municipality of residence. In municipalities served by water systems contaminated with volatile organic compounds, all individuals are exposed by some combination of inhalation, ingestion, and dermal absorption. The potential for exposure misclassification in our study resembled that of many occupational studies for which summary estimates of exposure are applied to all workers in a particular job description or location, irrespective of hot spots, personal protective equipment, or other personal factors that might influence individual exposure.

The relative risk estimates generated in our analyses were adjusted for individual data on age, sex, and race and weighted by population. Race minimally affected the estimates of the rate ratios in the regression analysis and was therefore not included in the report. Standard ecologic municipal socioeconomic indicators were examined qualitatively, but there were no notable differences between the exposure strata, and risk ratios were not adjusted for socioeconomic variables. In short, the most salient limitations of true ecologic designs did not apply to our study.

Ramlow and Bloemen also raise the issue of potential confounding in our study. Confounding occurs when a risk factor for disease is positively or negatively associated with an exposure of interest. Confounding can cause either overestimation or underestimation of the assoication between exposure and outcome. However, unless a confounder is a strong risk factor, the confounder must be strongly associated with the exposure of interest to significantly affect the results. Known strong risk factors for leukemia and NHL include certain genetic traits and DNA-repair enzyme deficiencies, exposure to benzene or radiation, and, for a few histologic types, infection with certain viruses. Smoking is a moderate risk factor for leukemia. There was no *a priori* reason to believe that these risks were differentially distributed among the exposure strata in our study. While we did not have information on smoking status (for malignancies in adults), neither do many occupational studies, including those cited in the letter from Ramlow and Bloeman.

Ramlow and Bloeman also raise the issue of the consistency of our findings with those from the occupational epidemiology and animal toxicology literatures. In evaluating the weight of evidence for potential public health hazards of PCE and TCE, we must consider that the general population includes subgroups that may be more sensitive than "healthy workers" to toxic agents. Additionally, Aschengrau et al.'s recent case–control study found strong associations between leukemia and PCE contamination of drinking water (*1*).

In carefully examining the occupational studies cited by Ramlow and Bloemen, we find that some of these studies, rather than contradicting our results, are consistent with our strongest finding. When duration